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INHERITANCE OF RESISTANCE TO THE PEA APHID IN ALFALFA HYBRIDS[1]

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THE PEA APHID *Macrosiphum pisi* (Kltb.) has at times caused severe damage to alfalfa (*Medicago sativa* L.) in certain areas in California, western Nevada, and elsewhere in the United States. In most of these sections, serious damage occurs at irregular intervals, the amount being largely dependent upon weather conditions during the winter and early spring. However, in the Antelope Valley of southern California, some damage has occurred regularly since 1924. During some seasons, 90 per cent of the fields have been damaged; during others, as few as 10 per cent have been affected. With a severe infestation, the first crop is a total loss, and the second is slow to develop because the plants are weakened by the aphids.

In the Antelope Valley and elsewhere, most of the known methods of insect control have been tried without pronounced success. Although grazing the fields immediately after the insects first appear has reduced losses, the isolation or breeding of resistant varieties seemed to offer the only satisfactory method of eliminating this aphid damage. Insect resistance in plants has been recognized for a long time. Blanchard and Dudley (1934)[5] were the first to report resistance to aphids in alfalfa. The genetics of resistance to insects in plants has engaged the attention of a number of investigators. The literature on the whole subject of insect resistance as related to crop plants was reviewed by Bigger (1943), Jones (1943), Dahms (1943), Blanchard (1943), and Painter (1943), in a symposium at the Annual Meeting of the American Society of Agronomy. Comparatively few cases were noted where a satisfactory genetic analysis of insect resistance has been made. No such case was reported for alfalfa.

A survey of badly infested fields in the Antelope Valley region revealed occasional plants that showed little or no damage. From 1930 to 1933, a num-

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ber of such plants were transferred to an alfalfa nursery near Sacramento. Some of these proved to be susceptible, but others were heterozygous for resistance as determined by progeny tests. The inheritance of resistance to the pea aphid for one of these plants, no. 334, has been studied in considerable detail. The results are reported in this paper.

PLANTS CHOSEN FOR STUDY

The alfalfa plants brought from the Antelope Valley were characteristically Common Chilean, the variety normally grown there. A number proved to be susceptible, while others were heterozygous for resistance. Plant no. 334 was selected for a detailed study because initial data suggested that it was segregating for a single factor for resistance. Additional data from the first and second selfed generations, which are equivalent to F_2 and F_3 generations in regard to the segregation of the character under consideration, failed to confirm this theory. Furthermore, the data did not suggest any other satisfactory genetic interpretation. Since the classification of some plants had been unsatisfactory, a change in technique was worked out, which we hoped would give more certain classification.

For further study, one of the resistant F_2 plants, no. 334–19, which, on the basis of F_3 and F_4 progeny tests, proved to be homozygous and highly resistant, was crossed reciprocally with a susceptible plant, no. 3344, of the same variety. From this cross, 503 F_2 plants were tested for resistance. Although an attempt was made to get selfed seed from all of these plants, sufficient seed for a progeny test was obtained on only 256 of them.

METHODS OF TESTING FOR RESISTANCE

Original Technique. In testing the progeny of plant no. 334 for aphid resistance, the seedlings were grown singly or in groups of not more than four in 10-inch pots and were covered with a cylindrical 18-mesh screen-wire cage. When the plants were an inch and a half or more tall and appeared to be growing rapidly, four or five female aphids were introduced into each cage. They were kept in the cage until a population large enough to damage the plant had accumulated or until the plants had demonstrated their resistance. The aphids multiplied rapidly on checks and other susceptible plants and did considerable damage in a week or 10 days. There was some variation in the rate of reproduction under different stages and conditions of plant growth, especially at different temperatures. Maximum populations developed only at temperatures that were high enough to insure the growth of succulent plants.

Aphids were unable to maintain themselves on the most resistant plants, although they usually bore several nymphs during the first few days. They lived from 4 to 15 days, depending upon the conditions of growth. At temperatures most suitable for rapid growth of the plants, they lived only a short time. The aphids reacted to feeding on resistant plants in a fairly uniform and characteristic way. After 1 or 2 days they became restless, some of them leaving the plants and crawling about the cage. The body color changed from pea green to blue green, and the abdomen became shrunken with a white fringe around the margin. The aphids died soon thereafter.

The fungus disease *Entomophthora aphidis,* common among aphids, was encountered from time to time. Aphids dead from this disease can be distinguished from those killed by feeding on resistant plants or from overcrowding.

Many plants showed an intermediate degree of infestation even under apparently favorable growth conditions. This ranged from a quite small population to one almost, if not entirely, equal to the lower limit obtained on the susceptible checks. Considerable uncertainty arose as to the proper classification of these plants.

Revised Technique. Before the progeny of resistant no. 334–19 × susceptible no. 3344 was tested, an effort was made to find means of assuring a more positive classification and thus permitting accurate differentiation between resistant and susceptible plants. Single plants were caged, and one second-instar nymph was introduced. The rate of nymph production and the length of the production period were used as measures of resistance. On plants of the resistant parent, second-instar nymphs usually failed to reach maturity in the normal time and upon maturity produced few nymphs or none at all. The length of life did not exceed 9 days. On plants of the susceptible parent, from 4 to 9 nymphs were produced per day for a period of 10 to 15 days. F_2 plants that appeared to be resistant on first test were reinfested to make certain that the classification was correct.

RESULTS OF PROGENY TESTS

The resistant parent no. 334–19 was selected from the progeny of no. 334, a heterozygous plant brought in from the Antelope Valley, on the basis of high resistance in the first, second, and third inbred generations. Second-instar aphids, caged singly with individual progeny plants of plant no. 334–19, usually failed to produce nymphs (table 1) but occasionally produced a small population during a period of 7 to 9 days. Under similar conditions, the progeny of susceptible parent plant no. 3344 produced an average of more than 5 per day during a period of 10 to 15 days.

Reciprocal crosses between resistant plant no. 334–19 and susceptible plant no. 3344 were made in 1935. Ten F_1 plants were tested and found to be almost as resistant as no. 334–19. These plants were placed in a large cage for the purpose of obtaining selfed seed. The flowers were tripped at 4-day intervals throughout the flowering period. The resulting set of seed was satisfactory.

Resistance in F_2. From this seed, 503 F_2 plants were tested individually for reaction to aphids; 262 were from one plant of no. 334–19 × no. 3344; and 241 were from one plant of the reciprocal cross. Aphid production ranged from 0.0 to 8.9 per day, with no difference between reciprocal crosses. The F_2 data are shown in table 1.

The revised technique differentiated clearly between the resistant and susceptible parents when average values are considered. However, when individual plants are taken into account, it will be seen that the number of nymphs on one resistant plant approached the lower limit of those found on susceptible plants. If all plants with an average of three or more nymphs per day are considered susceptible, as is indicated by the reaction of the susceptible parent plants, the data suggest a single dominant factor for resistance with

a ratio of 377 :126, where one of 377.25 :125.75 is expected. In almost every case where F_2 plants had from 3 to 4 nymphs in the first test, they were retested to verify their classification. Retesting was not done for the parents shown. When length of productive period is taken into account, it is believed that four aphids per day is a better value than three for separating resistant from susceptible plants. This value gives 408 resistant to 95 susceptible, figures very close to the 408.7 to 94.3 expected on the basis of the 13 :3 ratio that would result from one dominant and one recessive resistant factor. Neither ratio is borne out by the F_3 data. The segregation products show, however, that one dominant and one recessive gene for resistance are present.

TABLE 1

NYMPHS PRODUCED BY ONE APHID ON F_2 AND P_1 PLANTS

Nymphs produced per plant per day	F_2 plants	P_1 plants of resistant parent no. 334–19	P_1 plants of susceptible parent no. 3344
0.0	152	25	..
0.1–0.9	58	2	..
1.0–1.9	96	2	..
2.0–2.9	71	1	..
3.0–3.9	31	..	4
4.0–4.9	43	..	8
5.0–5.9	24	..	12
6.0–6.9	14	..	3
7.0–7.9	10	..	1
8.0–8.9	4	..	2

Percentages of Susceptible Plants in F_3 Families. An attempt was made to self all 503 F_2 plants in order to get seed for progeny tests. As is usual with alfalfa, considerable difficulty was experienced in getting seed. Some plants set no seed, and others produced too few to be useful. In all, 256 plants yielded enough seed to permit a progeny test. The number of plants in each progeny ranged from 8 to 70, with an average of 25. Some consideration was given to dropping families represented by fewer than 15 plants, but this procedure did not alter the final conclusions.

The percentage of susceptible plants in these 256 F_3 families, together with their F_2 classification according to the average number of nymphs produced on them, is shown in table 2. Plants were rated as susceptible if they had an average of four or more nymphs per day over a period of 10 or more days.

Analysis of Genes from F_3 Results. Among the F_3 populations, there are families homozygous for resistance and for susceptibility to aphid attack, as well as families that may be considered as segregating in 3 :1 and 1 :3 ratios (table 3). Such a distribution could result only from a dominant and a recessive gene. The F_2 classification was only partly correct, and the proportion of segregating and homozygous F_3 families is not in accordance with expectation on the basis of two independent genes.

Forty-four of the F_2 parents had four or more nymphs and were consequently classed as susceptible (table 3). These plants would be expected to

TABLE 2

F_3 FAMILIES CLASSIFIED BY PERCENTAGES OF SUSCEPTIBLE PLANTS

Average nymphs produced per day on F_2 parents	Number of F_3 families according to percentage of susceptible plants																				
	0.0 pct.	0.1 to 4.9 pct.	5.0 to 9.9 pct.	10.0 to 14.9 pct.	15.0 to 19.9 pct.	20.0 to 24.9 pct.	25.0 to 29.9 pct.	30.0 to 34.9 pct.	35.0 to 39.9 pct.	40.0 to 44.9 pct.	45.0 to 49.9 pct.	50.0 to 54.9 pct.	55.0 to 59.9 pct.	60.0 to 64.9 pct.	65.0 to 69.9 pct.	70.0 to 74.9 pct.	75.0 to 79.9 pct.	80.0 to 84.9 pct.	85.0 to 89.9 pct.	90.0 to 94.9 pct.	95.0 to 99.9 pct.*	Total
0	34	2	8	12	8	3	2	1	1	0	1	0	0	0	0	0	0	0	0	0	72
0.1–0.9	6	0	5	3	3	4	1	0	1	1	1	0	0	0	0	0	0	0	0	0	25
1.0–1.9	9	0	11	9	8	4	2	3	2	1	0	1	0	1	0	1	2	0	1	0	55
2.0–2.9	8	3	1	10	11	2	0	1	0	0	1	0	0	0	1	0	2	0	0	0	40
3.0–3.9	1	0	1	3	2	2	3	1	1	1	1	1	0	0	0	1	2	0	0	0	20
4.0–4.9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	3	0	9	16
5.0–5.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	11	12
6.0–6.9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	7	9
7.0–7.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	4
8.0–8.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3
Total	58	5	26	37	32	15	8	6	6	3	4	2	0	2	2	2	8	1	5	1	33	256

* No families in the percentage class 95.0 to 99.9.

TABLE 3

SEGREGATING AND HOMOZYGOUS F_3 FAMILIES, AND EXPECTATIONS BASED ON TWO INDEPENDENT AND ON TWO LINKED GENES

Both expectations based on one dominant and one recessive gene; 28.2 per cent crossover assumed with linked genes

F_2 classification, nymphs per day	Number of F_2 plants	Number of families in F_3 classification				
		Homozygous resistant	Segregating 3:1 and higher ratios	Segregating 1:3	Homozygous susceptible	Total
0	72	34	38	0	0	72
0.1–3.9	140	24	100	16	0	140
4.0 plus	44	0	0	11	33	44
Observed total	..	58	138	27	33	256
Expected, independent segregation	..	112	96	32	16	256
Expected, linked genes, 28.2 per cent crossover	..	95	102.1	25.9	33.0	256

give rise to F_3 families either homozygous for susceptibility or segregating at the ratio of one resistant to three susceptible. This proved to be the case, although they did not occur in the ratio of one homozygous to two segregating families, as would be expected on the basis of independent segregation, but in the ratio of 33 :11. Sixteen additional F_3 families segregated as 1 :3, these having 0.1 to 3.9 nymphs per day on their F_2 parents. The final ratio was thus 33 :27, which is still far from the 1 :2 ratio expected.

From the 140 F_2 parent plants, on which there had been an average production of from 0.1 to 3.9 nymphs per day, the resulting families might

reasonably have been expected to fall in the group segregating 3 :1 and higher ratios, with a few families homozygous for resistance (because occasional nymphs appeared on the resistant parent). In the main, these expectations were realized (table 3), although there were 16 families which segregated one resistant to three susceptible. (These were mentioned in the previous paragraph.)

Of these 16 heterozygous susceptible families, 11 were clearly evident. The other 5 were judged to belong to this group on the basis of the degree of resistance exhibited by the resistant segregates, a point that will be discussed later. Three of these 5 fell in the 45.0–49.9 per cent class and 2 in the 50.0–54.9 per cent class (table 2). The record of the 16 F_2 parents of these families is of interest: 1 fell in the 0.1–0.9 class, 6 in the 1.0–1.9 class, 4 in the 2.0–2.9 class, and 5 in the 3.0–3.9 class. Therefore, no point is indicated which clearly separates the homozygous and heterozygous susceptibles from other genotypes. While the criterion of four did screen out all the homozygous susceptible F_2 plants, it separated only about two thirds of the heterozygous susceptibles. If a lower point is taken, some other genotypes are included.

The 24 homozygous resistant families from the F_2 plants of this 0.1–3.9 group came from all classes of nymph production represented in it. They were distributed as follows: 6 from 0.1–0.9 class, 9 from the 1.0–1.9, 8 from the 2.0–2.9, and 1 from the 3.0–3.9 classes.

The 72 F_2 plants on which no nymphs were produced gave rise to families which were either homozygous for resistance or were segregating in the range of 3 :1 or higher ratios. This relation is in line with expectations, since the 10 F_1 plants tested were highly resistant. As the old technique was used with the F_1, no direct comparisons can be made.

The 27 families segregating one resistant to three susceptible plants (table 3) indicate the presence of a recessive factor for resistance. These families had 602 plants, of which 439, or 72.9 per cent, were susceptible. The resistant plants in these families were only intermediate in resistance. With one or two exceptions, all supported a population of nymphs, which in some cases approached that of the lower limits of susceptible plants.

There is ample evidence of the presence of a dominant gene for resistance, although families segregating in the ratio of three resistant to one susceptible cannot be distinguished from those segregating for a dominant and a recessive gene, thus giving a 13 :3 ratio.

The proportion of segregating to homozygous families observed is not in accordance with independent segregation, as may be seen in table 3. This is especially noticeable in the proportion of susceptible families to those segregating in the ratio of 1 :3. Linkage would bring about such a relation. Since only 58 of the 256 F_3 families, or 22.7 per cent, were resistant—hardly enough to satisfy the hypothesis of a single dominant resistant gene—we did not expect to find a crossover value that would permit a satisfactory fitting with all classes. There were 33 susceptible families in a population of 256, a relation which would result from a crossover of 28.2 per cent. The expected reaction of F_2 plants and segregation of F_3 families on this basis are given in figure 1, and the expected numbers for the population under consideration are shown in table 3.

While the divergence between observed and calculated numbers by the foregoing theory is statistically significant, it is not so great as that obtained from a number of other genetic theories considered. A few additional points follow which support the theory advanced.

	AB 14.1	**Ab** 35.9	**aB** 35.9	**ab** 14.1
AB 14.1	1 **AB/AB** 0.0199 F_2 *R* F_3 *R*	2 **Ab/AB** 0.0506 F_2 *R* F_3 *R*	3 **aB/AB** 0.0506 F_2 *R* F_3 25.0% *S*	4 **ab/AB** 0.0199 F_2 *R* F_3 2.0% *S*
Ab 35.9	5 **AB/Ab** 0.0506 F_2 *R* F_3 *R*	6 **Ab/Ab** 0.1289 F_2 *R* F_3 *R*	7 **aB/Ab** 0.1289 F_2 *R* F_3 16.4% *S*	8 **ab/Ab** 0.0506 F_2 *R* F_3 *R*
aB 35.9	9 **AB/aB** 0.0506 F_2 *R* F_3 25.0% *S*	10 **Ab/aB** 0.1289 F_2 *R* F_3 16.4% *S*	11 **aB/aB** 0.1289 F_2 *S* F_3 *S*	12 **ab/aB** 0.0506 F_2 *R* plus *S* F_3 75.0% *S*
ab 14.1	13 **AB/ab** 0.0199 F_2 *R* F_3 2.0% *S*	14 **Ab/ab** 0.0506 F_2 *R* F_3 *R*	15 **aB/ab** 0.0506 F_2 *R* plus *S* F_3 75.0% *S*	16 **ab/ab** 0.0199 F_2 *R* F_3 *R*

Fig. 1. Diagram of expected F_2 reaction and F_3 segregation for resistance (*R*) to aphids in alfalfa on the basis of a dominant (*A*) and a recessive (*b*) resistant gene, which are linked with a crossing over of 28.2 per cent. Value obtained from the 256 F_2 plants from which F_3 progenies in groups *aBAb* and *AbaB* were grown allows for the misclassification of about two thirds of the heterozygous susceptibles. Resistant plants in group *abab* almost without exception support a small population of aphids.

As pointed out earlier, the resistant plants in families segregating in the ratio of 1 :3 were somewhat intermediate in resistance. They supported nymph populations ranging from a few to numbers approaching the lower limits of the susceptible checks. Therefore, families of the genetype *aabb* (cell 16, fig. 1) should be intermediate, or without completely resistant plants. Four such families were found where 5.1 were expected.

The total number of F_3 families segregating out the lower percentages of

bunt expected from cells 3, 4, 7, 9, 10, and 13 conforms very well to the hypotheses under consideration. In these, there were 578 susceptible plants out of a total population of 3,674, or 15.7 per cent. The average per cent expected from these families, according to figure 1, is 17.1.

The homozygous resistant families are too few to support the above hypothesis. In fact, as noted earlier, there are hardly enough to satisfy the hypothesis of a single dominant gene. The question arises as to whether this is a chance deviation or a result of incorrect classification of some of the families. There is a corresponding excess of families among those classified as segregating for 3 :1 and higher ratios. It will be recalled that one resistant parent plant (table 1) had a fairly high population of nymphs. It was also pointed out that some plants, homozygous for the recessive gene only, supported populations approaching the lower limits of the homozygous susceptible plants. This fact suggests that there might have been some incorrect classifications, especially in families arising from *Aabb*, genotypes where one fourth of the population would be intermediate. However, every effort was made to guard against incorrect classifications by retesting susceptible plants in segregating families if there was any doubt about their susceptibility. Certainly the entire deficit was not likely to be accounted for in this way. There is no evidence that the 256 families did not represent a random sample. Nor is there reason to believe that homozygous resistant plants did not seed as readily as others.

DISCUSSION AND CONCLUSIONS

The development of insect-resistant crops presupposes the discovery of resistant varieties or selections. If it becomes necessary to breed a variety suited to some particular area, a knowledge of the genetics of resistance is essential to a well-planned and intelligent breeding program.

A necessary adjunct to a study of resistance is a suitable method of testing that will not only distinguish between resistant and susceptible plants but will also distinguish between different levels of resistance. The procedure used here—that of caging one second-instar nymph of the pea aphid with each plant—was satisfactory for detecting homozygous susceptible plants. Only about one half of the heterozygous susceptible plants resulting from the action of a recessive resistant gene, however, fell in the susceptible group in F_2, as determined by progeny tests. It was felt that the above procedure gave a fairly accurate classification of F_3 families.

The F_3 data clearly indicate the presence of a recessive resistant gene. At least one, and probably only one, dominant gene also is present. This proportion of homozygous to heterozygous families suggests the presence of linkage with a crossover value of about 28 per cent indicated.

For no apparent reason there was a deficiency of homozygous resistant families and a corresponding excess of heterozygous families. This divergence could not be explained on the basis of linkage or any other genetic theory tried.

Comparatively few genetic studies have been made with alfalfa, and frequently those have been a by-product of some other problem. For the most part, the data have not yielded the simple genetic ratios usually found in

other crops. The chromosome number in common alfalfa indicates that it is a tetraploid. Recently Tysdal, Kiesselbach, and Westover (1942) suggested that it is an autotetraploid, and that some segregation products found may be explained on the basis of random chromatid segregation. This theory did not apply in the case of aphid resistance.

Along with this genetic study, we have made satisfactory progress in breeding an aphid-resistant alfalfa of the Common Chilean type. Although the project is not yet completed, there is every reason to believe it will be successful.

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INHERITANCE OF RESISTANCE TO SCALD IN BARLEY[1]

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SCALD, CAUSED BY *Rhynchosporium secalis* (Oud.) Davis, is a leaf disease of barley that is of considerable importance in California. In 1936, when this work was undertaken, none of the varieties grown commercially in this state had a high degree of resistance, and Atlas, the most widely grown variety, was highly susceptible. With the discovery of the resistant varieties reported by Riddle and Suneson (1948),[4] it seemed desirable to study the inheritance of resistance to scald and to inaugurate a breeding program to develop a resistant strain of Atlas.

VARIETIES AND SELECTIONS USED

Four resistant varieties and selections—La Mesita, selection Calif. No. 1311, Trebi, and Turk (C. I. 5611–2)—were used in crosses with susceptible Atlas and with each other. A brief history of each variety is given below.

La Mesita, Calif. No. 1002 originated as a plant selection from California Mariout, which in turn was introduced from Egypt. It was grown commercially for a time in Santa Barbara County, California.

Calif. No. 1311 is a selection from composite cross C. I. 5461, which has been described by Harlan, Martini, and Stevens (1940). It was selected at the Aberdeen Experimental Substation, Aberdeen, Idaho, from the above composite. Calif. No. 1311 was first grown at Davis in 1937 but has not been grown commercially.

Trebi, C. I. 936 originated as a selection from a variety brought to the United States from the south shore of the Black Sea. It was released from the Aberdeen Experimental Substation in 1918 (Harlan and Martini, 1936). This variety is not grown commercially in California.

Turk, C. I. 5611–2, according to Dr. G. A. Wiebe,[5] traces to material collected in 1928 in northeastern Turkey and introduced into the United States in 1930. It is a very late, two-rowed, rough-awned, weak-strawed type, used only experimentally in this country. Because of its high resistance to scald, it was used in the backcross program with Atlas to introduce resistance into that variety.

Atlas, C. I. 4118 (Harlan and Martini, 1936) is the variety of malting barley most widely grown in California. It originated as a pure-line selection made from Coast barley in 1917. Coast was introduced into California by the early Spanish settlers at the time the missions were established in this state. It is very susceptible to scald.

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RANGE OF INFECTION

Natural Infection in the Field. The reaction of barley varieties and hybrids to scald was studied in the field and to some extent in the greenhouse. Field plantings were made early (late October or early November) contiguous to barley stubble, conditions which generally resulted in adequate natural infection. F_2 and F_3 seeds were spaced 4 inches apart, in rows 1 foot apart and 16 feet long. This spacing generally resulted in more than forty plants that could be examined in place. They were classified when scald development apparently had reached the maximum, usually a few days before heading. Five classes of infection were set up: 0, highly resistant; 1, resistant; 2–3, weak resistance; and 4, susceptible. As with many such arbitrary classifications, the class limits are hard to define, yet they are usable. The presence of other diseases, particularly of net blotch, frequently made field classifications difficult and uncertain.

Artificial Inoculation in the Greenhouse. Classification of greenhouse-grown plants artificially inoculated with scald in the seedling stage was highly satisfactory. Those varieties exhibiting a slight infection of scald at the near-heading stage in the field generally were completely resistant at the time of classification in the greenhouse. Susceptible checks showed a heavy infection.

In the greenhouse, seeds were spaced 1 inch apart, in rows 30 inches long and 3 inches apart. The soil benches were filled to a depth of 6 inches. This gave populations of from 25 to 30 plants per row. About two weeks after emergence, when the third leaf was well started, the plants were sprayed with a spore suspension of the scald fungus. The benches were covered for 48 hours with a cheesecloth tent; the humidity was maintained near 100 per cent and the temperature close to 70° F. Spore suspensions were made from cultures 8 days old. Plants were ready for classification about two weeks after inoculation.

A mixture of six different cultures was used for inoculations in the greenhouse. No attempt was made to determine whether these represented physiological races differing in pathogenicity. Varietal reactions indicated that these cultures represented the same race or mixture of races active in the field at that time.

Hybrid populations involving the varieties mentioned above will be reported in turn.

VARIETAL REACTIONS TO SCALD

The reactions to scald of the several varieties and selections are given in table 1. Turk (C. I. 5611–2) and the lines extracted from Turk × Atlas showed no scald either in the field or in the greenhouse. Calif. No. 1311, Trebi, and La Mesita, all showed some scald in the field under some conditions. They also developed this disease in the greenhouse when allowed to grow beyond the seedling stage. Atlas generally had a type-4 infection.

Atlas × La Mesita. Atlas was highly susceptible when grown either in the field or in the greenhouse. La Mesita was resistant, showing a range of 0–2 type infection in the field but always 0 in the greenhouse at the normal time of classification.

In the field the F_1 reaction was very similar to that of La Mesita. The presence of net blotch as well as of some scald on La Mesita and other resistant plants made the classification of field-grown F_2 plants unsatisfactory. However, an attempted classification of 204 F_3 rows as resistant, segregating, and susceptible was fairly successful. Later, 39 of these rows of about 30 plants each were tested under greenhouse conditions. It was found that most of them had been classified correctly in the field, where the distribution by rows had been 57 resistant: 96 segregating: 51 susceptible (table 3). This led to a

TABLE 1

REACTION OF BARLEY PARENT VARIETIES AND SELECTIONS TO SCALD INFECTION

Grown in the field at Davis, 1937–1943, and in the greenhouse in 1943

Variety or selection	California no.	Infection type on 0–4 scale									
		1937 4/24	1938 3/18	1939 4/1	1940 3/23	1941 3/27	1942		1943 4/10	Greenhouse 1943	
							3/23	4/3		1/29	2/13
Turk, C.I. No. 5611-2	1315	0	0	0	0	0	0	0	0	0	0
P-4229*	1312	..	..	..	..	..	0	0	0	0	0
P-4232*	1313	..	..	..	..	..	0	0	0	0	0
P-4233*	1328	..	..	..	..	..	0	0	0	0	0
P-4236*	1314	..	..	..	..	..	0	0	0	0	0
Calif. No. 1311	1311	..	0	0	0	0	0–2	0–4	1	0	1
Trebi, C.I. No. 936	1004	0	0	0	1	1	0	0–3	2	0	2
La Mesita	1002	0	1	2	1	1	2	tr.	1–2	0	2
Atlas, C.I. No. 4118	....	4	4	4	4	4	4	4	4	3–4	4

* Single-dominant-factor resistant types derived from Turk × Atlas.

tentative conclusion that La Mesita differed from Atlas by a single gene for resistance to scald.

In the greenhouse, no difficulty was experienced in classifying the completely resistant F_2 plants. Out of a total of 815, there were 614, or 75.3 per cent, having the 0 reading like La Mesita (table 2). The other 201 plants had a range of disease intensity from 1 to 4, which was the same as the range of intensities encountered in Atlas (2, 2, 18, 134 plants with reading of 1, 2, 3, and 4 types). Thus, there were similar proportions of the several types in each population.

Thirty-nine F_3 rows from F_2 plants that had not been classified for scald resistance were grown in the greenhouse (table 3). These gave 9 resistant: 24 heterozygous: 6 susceptible, where 9.8 :19.4 :9.8 were expected on the basis of the single factor postulated above. Thus, all the data agree in showing that La Mesita differs from Atlas by a single gene for resistance to scald.

If dominance is based on complete resistance, it would have to be considered somewhat intermediate in the field, although the F_1 plants were as resistant as the resistant parent. In the greenhouse, both the La Mesita and the heterozygous plants were completely resistant at the time readings were made. Later, however, they both developed a little scald.

TABLE 2

DISTRIBUTION OF F_2 PLANTS, FROM CROSSES OF ATLAS WITH RESISTANT VARIETIES AND SELECTIONS

Grown at Davis, 1939–1943

Cross	Classified on 0–4 scale of infection type										
	Number of plants					Total	Per cent of plants				
	0	1	2	3	4		0	1	2	3	4
Atlas × La Mesita*	614	1	2	33	165	815	75.3	0.1	0.2	4.0	20.2
Atlas × Calif. No. 1311*¶	235	6	1	10	70	322	73.0	1.9	0.3	3.1	21.7
Atlas × Calif. No. 1311*¶	137	22	22	22	46	249	55.0	8.8	8.8	8.8	18.5
Atlas × Trebi*¶	311	8	8	18	84	429	72.5	1.9	1.9	4.2	19.6
Atlas × Trebi*¶	172	36	16	13	53	290	59.3	12.4	5.5	4.5	18.3
Turk × Atlas†¶	1479	7	42	10	208	1746	84.7	0.4	2.4	0.6	11.9
Turk × Atlas†¶	821	7	35	18	142	1023	80.3	0.7	3.4	1.8	13.9
Atlas × P-4229†‡	127§	..	..	..	42	169	75.1	....§	....	....	24.9
Atlas × P-4232†	121§	..	..	..	45	166	72.9	....	....	....	27.1
Atlas × P-4233†	145§	..	..	..	55	200	72.5	....	....	....	27.5
Atlas × P-4236†	138§	..	..	..	37	175	78.9	....	....	....	21.1
Atlas × P-42683*	399	..	7	18	114	538	74.2	0.0	1.3	3.3	21.2
Atlas × P-42709*	409	2	5	16	96	528	77.5	0.4	0.9	3.0	18.2

* All data on greenhouse-grown material.
† Data from both field-grown and greenhouse-grown material.
‡ P-4229 to P-42709 are homozygous derivatives from the cross, Turk × Atlas.
§ 1, 2, and 3 classification types not used for these crosses.
¶ Data taken on two dates.

TABLE 3

SUMMARY OF F_2 AND F_3 DATA FROM THE CROSS, ATLAS × LA MESITA

Grown at Davis in 1940 and 1943

	Resistant (type 0)	Segregating	Susceptible (types 1–4)	Chi^2	*P*
Field-grown F_3 (rows)					
Observed	57	96	51	.....	.....
Expected (with single gene difference)	51.0	102.0	51.0	1.0588	0.5–0.7
Greenhouse-grown F_2 (plants)					
Observed	614	..	201	.....	.....
Expected (with single gene difference)	611.3	..	203.7	0.0477	0.8–0.9
Greenhouse-grown F_3 (rows)					
Observed	9	24	6	.....	.....
Expected (with single gene difference)	9.8	19.4	9.8	2.6295	0.2–0.3

Atlas × Calif. No. 1311. Calif. No. 1311 has sometimes shown light infection in the field; the F_2 classification, therefore—as was the case with La Mesita—was unsatisfactory for genetic interpretation.

F_2 populations were grown in the greenhouse and classified on two different dates. The data are reported separately in table 2. The differences in the percentages of plants falling in each infection type are not readily explain-

able. The second set of data reported was obtained under the heaviest infection experienced in the greenhouse. Apparently a greater number of resistant plants showed type 2 and 3 reaction than was the case under lighter infection, although Calif. No. 1311 showed no disease in either case. Both the F_2 population under consideration and the F_3, to be discussed later, trace to the same F_1 plant.

Seventy-two F_3 rows from F_2 plants that had not been classified for scald resistance were grown in the greenhouse, but at a date different from that of either of the F_2 populations. The F_3 data indicate the presence of two

TABLE 4

ANALYSIS OF F_3 DATA FROM THE CROSS, ATLAS × CALIF. NO. 1311, AS RELATED TO F_2 GENOTYPES*

F_2 genotype	Expected F_3 reaction	Number of F_3 rows		Number of F_3 plants					
				Total	Observed distribution in infection types				
		Observed	Expected		0	1	2	3	4
AABB, *AABb*, *AAbb*	All resistant (0 type)	20	18.0	389	389	0	0	0	0
Aabb	3 (0 type): 1 (†0–4 type)	10	9.0	194	145	22	17	7	3
aabb	Mostly 2 type. Range 0–4	4	4.5	77	1	11	43	17	5
AaBB	3 (0 type): 1 (3–4 type)	7	9.0	128	99	0	0	15	14
AaBb	12 (0 type): 1 (0–4 type): 3 (3–4 type)	21	18.0	406	248	27	26	50	55
aaBb	1 (0–4 type): 3 (3–4 type)	6	9.0	112	0	2	23	52	35
aaBB	All susceptible (3–4 type)	4	4.5	73	0	0	1	25	47
AAbb	(Resistant parent–Calif. No. 1311)	..	....	38	38	0	0	0	0
aaBB	(Susceptible parent–Atlas)	..	....	139	0	0	2	21	116

* It is assumed that the presence of the dominant gene *A* in an F_3 plant results in 0 type reaction and that the recessive *bb* genotype imparts resistance but permits an infection range of 0 to 4 types with most plants in the 1 to 3 groups.

† Actual expected distribution same as for the *aabb* genotype.

independent genes for resistance to scald in Calif. No. 1311, one of which is dominant and the other recessive. If it is assumed that the dominant gene *A* imparts complete resistance and that the recessive gene—when homozygous *bb*—permits the full 0 to 4 range of infection with a mode at 2, some of the F_2 genotypes can be determined quite accurately by the behavior of F_3 rows. In table 4, an attempt is made to group rows of comparable segregation and to assign appropriate genotypes to them. For example, the F_3 rows in which all plants are scald-free are assumed to trace to one of the three genotypes homozygous for *A* (*AABB*, *AABb*, or *AAbb*). While not all F_3 rows may be assigned with as great certainty as these, a reasonably good case can be made for all. The close agreement of observed with expected numbers of rows demonstrates the probable correctness of the assumption that Calif. No. 1311 differs from Atlas in one dominant and one recessive gene for resistance to scald.

Atlas × Trebi. The field reaction of Trebi has been similar to that of Calif. No. 1311 in showing occasional light infection. As with the two crosses previ-

TABLE 5

ANALYSIS OF F_3 DATA FROM THE CROSS, ATLAS × TREBI, AS RELATED TO F_2 GENOTYPES*

F_2 genotype	Expected F_3 reaction	Number of F_3 rows		Number of F_3 plants					
				Total	Observed distribution in infection types				
		Observed	Expected		0	1	2	3	4
AABB, *AABb*, *AAbb*	All resistant (0 type)	35	33.8	610	610	0	0	0	0
Aabb	3 (0 type): 1 (†0–4 type)	14	16.9	247	198	6	21	15	7
aabb	Mostly 1-3 type. Range 0-4	6	8.4	95	9	25	24	28	9
AaBB	3 (0 type): 1 (3–4 type)	52	50.6	894	661	12	22	63	136
AaBb	12 (0 type): 1 (0–4 type): 3 (3–4 type)								
aaBb	1 (0–4 type): 3 (3–4 type)	23	16.9	395	27	35	80	117	136
aaBB	All susceptible (3–4 type)	5	8.4	87	0	0	3	13	71
AAbb	(Resistant parent–Trebi)	..	....	40	40	0	0	0	0
aaBB	(Susceptible parent–Atlas)	..	....	78	0	0	0	0	78

* It is assumed that the presence of the dominant gene *A* in an F_2 plant results in 0 type reaction and that the recessive *bb* genotype imparts resistance but permits an infection range of 0 to 4 types with most plants in the 1 to 3 groups.

† Actual expected distribution same as for the *aabb* genotype.

TABLE 6

SUMMARY OF F_2 DATA FROM CROSSES OF ATLAS WITH SIX DERIVATIVES FROM TURK

Grown in the field and in the greenhouse at Davis in 1943

Cross	Observed		Expected (with single gene difference)		Chi^2	*P*
	Resistant (type 0)	Susceptible (types 1–4)	Resistant (type 0)	Susceptible (types 1–4)		
Atlas × P-4229	127	42	126.8	42.2	0.0127	0.9–0.95
Atlas × P-4232	121	45	124.5	41.5	0.3936	0.5–0.7
Atlas × P-4233	145	55	150.0	50.0	0.6667	0.3–0.5
Atlas × P-4236	138	37	131.2	43.8	1.4081	0.2–0.3
Atlas × P-42683	399	139	403.5	134.5	0.2008	0.5–0.7
Atlas × P-42709	409	119	396.0	132.0	1.7071	0.1–0.2
Combined data	1339	437	1332.0	444.0	0.1472	0.7–0.8

ously discussed, field-grown F_2 populations have not yielded data satisfactory for genetic interpretation.

The behavior of greenhouse-tested F_2 populations is very similar to that of Atlas × Calif. No. 1311 (table 2). The second set of data was obtained under the same heavy infection reported for Atlas × Calif. No. 1311.

F_3 data from 135 rows grown in the greenhouse are reported in table 5. An analysis comparable to that used for Calif. No. 1311 indicates that Trebi also differs from Atlas in one dominant and one recessive gene for resistance to this disease.

TABLE 7

DISTRIBUTION OF F_3 ROWS FROM THE CROSS, TURK × ATLAS, IN 5 PER CENT CLASSES OF SCALD INFECTION

Grown in the field and in the greenhouse at Davis, 1940 to 1942

Per cent of plants susceptible to scald (types 3 and 4)	Number of rows	Per cent of rows	Per cent of plants susceptible to scald (types 3 and 4)	Number of rows	Per cent of rows
0	164	30.5	50.0–54.9	1	0.2
0.1– 4.9	26	4.8	55.0–59.9	0	0.0
5.0– 9.9	38	7.1	60.0–64.9	1	0.2
10.0–14.9	48	8.9	65.0–69.9	0	0.0
15.0–19.9	58	10.8	70.0–74.9	0	0.0
20.0–24.9	55	10.2	75.0–79.9	1	0.2
25.0–29.9	35	6.5	80.0–84.9	1	0.2
30.0–34.9	15	2.8	85.0–89.9	0	0.0
35.0–39.9	13	2.4	90.0–94.9	8	1.5
40.0–44.9	6	1.1	95.0–99.9	12	2.2
45.0–49.9	0	0.0	100.0	56	10.4
			Total	538	100.0

Turk (and Its Derivatives) × Atlas. Turk and its single-dominant-gene extractions have shown complete resistance to scald both in the field and in the greenhouse. Because the genetics of resistance to scald in Turk × Atlas has proved to be complex and therefore difficult to analyze, the six extracted resistant lines will be discussed first. Six homozygous plants—P-4229, P-4232, P-4233, P-4236, P-42683, and P-42709—were obtained from three F_3 rows of Turk × Atlas, which were segregating 3 resistant to 1 susceptible plant. Each of these lines gives clear-cut monohybrid ratios in F_2 when crossed with Atlas (table 6). No difficulty was experienced in classifying plants either in the field or in the greenhouse. The crosses necessary to establish the relationship among the genes of these six lines have been studied. All proved to have the same gene (table 8).

The distribution of 538 F_3 rows of the Turk × Atlas cross into 5 per cent classes of scald infection is shown in table 7. These were grown and classified

TABLE 8

THE F_2 OF CROSSES BETWEEN RESISTANT VARIETIES AND SELECTIONS

Grown in the greenhouse at Davis in 1945

Hybrid	Number of plants		Hybrid	Number of plants	
	Total	Susceptible		Total	Susceptible
La Mesita × Calif. No. 1311	499	0	Trebi × Turk	237‡	0
La Mesita × Trebi	302*	0	Trebi × P-4229	194	0
La Mesita × Turk	333†	0	Trebi × P-4232	284	0
La Mesita × P-4229	258	0	P-4232 × P-4233	221	0
La Mesita × P-4232	273	0	P-4232 × P-4236	593	0
Calif. No. 1311 × Trebi	71	0	P-4233 × P-4236	359	0
Calif. No. 1311 × Turk	32	0	P-4236 × P-42683	352	0
Calif. No. 1311 × P-4229	201	0	P-4236 × P-42709	337	0
Calif. No. 1311 × P-4232	267	0	P-42683 × P-42709	269	0

*, †, ‡, further verified by 41, 42, and 42 F_3 rows, respectively, all of which were scald-free.

as follows: (1) In 1940 all plants in 320 field-grown rows were classified on the 0 to 4 scale of infection. All susceptible rows as well as those showing a high percentage of susceptible plants were duplicated and rechecked in 1942. (2) In 1941, all plants in 218 field-grown rows were classified as susceptible, resistant (0 type), or doubtful. All doubtful plants were carried into the F_4 in 1942 and there classified as resistant or susceptible. Of the 218 F_3 rows, 170 were duplicated in the greenhouse in 1942.

When only the 3 and 4 types of infection were considered as susceptible, all distributions were essentially alike and therefore have been combined in table 7. This distribution cannot be explained on the basis of the single gene present in the lines extracted from Turk × Atlas and reported above. There are a few lines with from 50 to 90 per cent susceptible plants, suggesting the presence of a recessive gene. Numerous attempts were made to explain the distribution obtained by the use of additional dominant and recessive genes with and without linkage. In every case, there were serious discrepancies for some parts of the data. It seems best, therefore, to await further data before presenting conclusions as to the genetic constitution of Turk with reference to resistance to scald.

Crosses of Resistant Varieties. Data on the relation of the dominant gene found in La Mesita, Calif. No. 1311, Trebi, and monogenic lines from Turk × Atlas were obtained in the greenhouse and are given in table 8. Not all the crosses between all the resistant varieties were available for study, but a sufficient number was investigated to demonstrate clearly their gene relationship. In no case was a susceptible F_2 plant found. Forty-one, 42, and 42 F_3 rows of La Mesita × Trebi, La Mesita × Turk, and Trebi × Turk, respectively, were grown without a susceptible plant being found. The above varieties and selections appear, therefore, to have a single dominant gene in common.

Level and Transmission of Resistance. The fact that the dominant gene common to all the varieties under consideration does not confer equal resistance on all of them and does not transmit equal resistance to progeny is difficult to interpret. Actually, the varieties appear to fall into two groups as far as level and transmission of resistance are concerned. Turk and its derivatives make up one group, while Calif. No. 1311, Trebi, and La Mesita constitute the other. Three possible explanations are suggested: (1) These two groups of varieties have different modifying gene complexes. In this connection it should be pointed out that this gene from Turk went through seven backcrosses with Atlas in developing the scald resistance of Atlas 46[6] without losing any of its effectiveness in controlling this disease. (2) Two closely linked genes should be considered. Of the 5,082 F_2 plants included in table 8, only 1,748 plus 42 F_3 rows are useful in determining this point. This small number of plants will not eliminate linkage. (3) Different alleles of the same gene would account for the differences.

[6] To be reported later.

DISCUSSION

The classification of plants for scald resistance was difficult when other leaf diseases were present, particularly net blotch. When greenhouse-grown plants were artificially inoculated, this trouble disappeared. Therefore, for genetic studies the data collected in the greenhouse were much more useful than field data. However, the study of varietal reaction and the breeding of scald-resistant Atlas 46 were successfully carried out in the field.

La Mesita differs from susceptible Atlas by a single dominant or near-dominant gene for resistance to scald.

The data presented indicate that both Trebi and Calif. No. 1311 have a dominant and a recessive gene for resistance to scald. Since Trebi was one of the parents of a composite cross, from which Calif. No. 1311 was derived, resistance of the latter could trace to Trebi. The dominant gene in both varieties is identical with the one in La Mesita.

Turk has been the source of the highest type of resistance studied thus far. Six single-gene extractions from Turk × Atlas have shown good 3:1 ratios in F_2, with little difficulty in classification either in the field or in the greenhouse. The presence in Turk of one or more additional genes for resistance to this disease has been established from extensive studies involving F_2 to F_4 generations of Turk × Atlas. Thus the number and their mode of inheritance have not been established accurately.

The single gene extracted from Turk appears to be identical with the dominant gene in La Mesita, Calif. No. 1311, and Trebi. Turk and its extractions have been completely resistant in the field, whereas the other three varieties may sometimes show a moderate amount of the disease. These differences may be due to modifying genes, different but closely linked genes, or possibly multiple allels.

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PHYSIOLOGIC AND GENETIC STUDIES WITH THE STRIPE DISEASE IN BARLEY[1, 2]

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THE DISCOVERY of a male-sterile barley and its use to facilitate floral infection with spores of *Helminthosporium gramineum* Rabh. (Suneson and Houston, 1942)[4] has provided a new method for study of the stripe disease in barley. The experiments reported in this paper were made possible by adaptations of this new technique for inducing infection.

To establish facts that will aid in breeding varieties resistant to stripe is an objective important for California; stripe was found in 12 of 53 fields surveyed in 1943 (Suneson and Santoni, 1943*b*) and in 49 of 99 fields surveyed in 1949 (Suneson, 1949). The continued presence of stripe in such proportions does not discredit recommended seed treatments for control but certainly does show that many farmers either do not treat seed at all or do not use seed treatments properly.

Distribution and Description of Stripe Disease. A general review of the literature (Dickson, 1939) shows that barley stripe is distributed throughout the humid and semihumid temperate regions of the world. Only rarely are more than 20 per cent of the plants in a field affected.

Diseased plants show yellow stripes soon after tillering. These ultimately darken. At heading time the entire plant darkens and becomes brittle, rarely producing heads or seed. At this period wind-borne spores initiate the floral infections, which are expressed in the succeeding crop.

Fundamental information regarding the life history of stripe and the role of environment in its development and expression is rather limited. Most investigators have depended upon natural infections, which at best produce only 10 to 60 per cent of diseased plants (Suneson and Santoni, 1943*a*). Seed treatment has reduced the severity of the disease.

Techniques for Inducing Infection. The technique followed in this paper (Suneson and Houston, 1942) provides positive deposition of spores within the hulls at the flowering period. An alternative controlled method of inoculation involves germinating seeds in direct contact with a mycelial mass of the organism grown on a culture medium. Shands and Arny (1944) using this method obtained satisfactory infections in only 5 out of 8 years. With it, a large number of culture plates is necessary. Moreover, the germinated seeds must have careful handling and timely planting. Recent California work has produced a variation in the method whereby the organism can be induced to sporulate in culture (Houston and Oswald, 1946). Furthermore, germination in contact with growing cultures apparently does not require

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[4] See "Literature Cited" for citations referred to in text by author and date.

highly specific time intervals and temperatures for infection, and seeds can be dried for 10 days following germination without harm to the organism (Houston and Oswald, 1948).

Floral or seedling infection does not assure full expression of the disease. A temperature of 15° C, or lower, during the period of emergence and a soil that is less than 40 per cent saturated have been reported as most conducive to stripe development (Leukel, Dickson, and Johnson, 1933). Vernalization for 38 days at 31° to 34° F resulted in a sharp increase in stripe over paired plantings of nonvernalized seed (Åberg, 1945).

Preliminary Work on Resistance to Stripe. Several workers have observed resistance to stripe. The preliminary California attempt to determine a genetic basis for resistance was based entirely on F_1 response (Suneson and Santoni, 1943*a*). In this work, the dominance of resistance in Hannchen, the intermediate reaction in Trebi, and the dominance of susceptibility in Club Mariout were indicated. A subsequent genetic analysis (Arny, 1945*a*) indicated a 3-factor difference in Oderbrucker × Brachytic. The resistance in Brachytic was different from that in Lion. Similarly, the susceptibility of Oderbrucker differed from that in Colsess IV.

Races of the Organism. Physiologic specialization of the stripe fungus has been noted by several workers. The basis for their differentiation has involved variances in spore development, in effect on growth, or in degree of infection. The greatest difference reported involves the variety Atlas. In tests conducted in Wisconsin for three years, Atlas developed no stripe (Shands and Arny, 1944), although it has long been known to be susceptible in California. This California susceptibility is not so complete, however, as for some other varieties (Suneson and Santoni, 1943*a*). A stripe culture capable of infecting Atlas has recently been reported from Wisconsin (Arny, 1945*b*).

ESTABLISHING AND EVALUATING INFECTIONS

Inoculation by the method reported in this paper differs from natural field infection only in controlling the time of deposition and the placement of the spores within the flower. Male sterility with its attendant open flowers is requisite. Fertilization and inoculation are consecutive or practically concurrent dusting operations (Suneson and Houston, 1942). The method is applicable to the F_1 generation from crosses with male-sterile, to backcrosses to male-sterile, or to segregates from crosses or backcrosses expressing male sterility.

Except for special race studies, a presumed single culture (no. 3) of stripe has been used throughout the experiments. It was propagated from season to season on male-sterile × Atlas. Other cultures for race studies have been similarly maintained. The only safeguard against contamination involved bagging after inoculation.

Plants were classified as diseased or healthy, depending on the presence or absence of spore-producing stripes on the leaves. In some seasons all diseased plants died before producing viable seeds, while in others a few plants produced viable seed on some tillers. Such seasonal variations were ignored in evaluating infections, however. Plants were treated as healthy or as totally diseased.

RESULTS

Effect of Humidity at Time of Flowering. Either a semihumid condition or wetting of spores is considered necessary for producing the stripe disease by floral infection (Dickson, 1939). In California and other parts of the arid Southwest, rains between flowering date and maturity are infrequent. Although the humidity may sometimes be high at night, it is generally very low from about noon until sunset. Nevertheless, natural infections may produce as much as 70 per cent stripe (Suneson, 1949).

TABLE 1

CHRONOLOGY ON THE DEVELOPMENT OF STRIPE FROM PAIRED HUMIDITY TREATMENTS IN THE CROSS *msms* × ROJO

Grown at Davis, California*

Pair No.	Dry spore inoculation and continuous low humidity after inoculation					Spores wet and high humidity maintained for 6 hours after inoculation				
	Number of plants established from spike	Number of plants with stripe on:†			Total stripe per cent	Number of plants established from spike	Number of plants with stripe on:†			Total stripe per cent
		April 10	April 18	May 11			April 10	April 18	May 11	
1	28	14	0	0	50	29	22	3	0	86
2	33	18	2	0	61	39	19	2	0	54
3	28	22	1	0	82	42	21	2	0	55
4	30	18	0	0	60	14	9	0	0	64
5	29	20	1	0	72	48	25	3	1	60
6	30	15	3	2	67	46	29	3	1	72
7	32	23	0	1	75	33	20	0	1	64
8	27	24	0	2	96	29	15	4	1	69
9	28	21	1	0	79	32	22	3	2	84
10	27	12	3	0	56	.	.	.	.	.

* Seeds were planted November 28 and plants emerged December 15, 1944. Plants were 10% headed on April 18, 1945.

† The plants were removed when classified as diseased.

The experiment reported in table 1 involved paired heads with contrasting treatments during the first 6 hours after pollination and inoculation. Subsequently, the natural closing of the hulls and the protective covering of the bag on each spike are believed to have cushioned the night–day variations in humidity. Pollination and inoculation were made at 11 a.m. The wet treatment included replenishment of water in the thick covering of absorbent paper around the spike each hour for 6 hours. Dry dusting of spores—the general procedure—produced essentially the same stripe infections as did the wetting of spores.

Certain data in table 1 are typical of all the experiments discussed. The number of plants established ranged from 14 to 48 per pollinated and inoculated spike. These plants had a range of from 50 to 96 per cent stripe infection. Maximum sporulation occurred just prior to flowering. The first stripe symptoms were observed on March 2; 90 per cent of the total ultimate stripe was evident by April 10. When the remaining nondiseased plants were 10 per cent headed on April 18, an additional 7 per cent of the total expressed stripe was

observable. On May 11, the seasonal stripe development was complete, the final 3 per cent occurring after the start of heading.

Effect of Stripe on Germination. Stripe infection of seeds apparently does not reduce germination. No significant differences in stand were noted during two seasons, in tests between paired uninoculated and inoculated seeds of several diverse stocks. In the first season only 63 and 64 per cent, respectively, of the seeds produced plants, whereas in the second, 93 per cent stands of both groups were obtained.

Uniform germination is a requisite in the malting of barley. A commercial maltster gave a "satisfactory" rating to three farm lots of 1948-crop barley, which produced 50–60 per cent stripe when sown at Davis without seed treat-

TABLE 2

EFFECT OF STRIPE-INDUCED HULL DISCOLORATION ON PLANT ESTABLISHMENT AND STRIPE PERCENTAGE

Field tests at Davis, California, 1946

Cross	Dark-colored hulls			Bright hulls		
	Number of seeds planted	Per cent established	Per cent stripe	Number of seeds planted	Per cent established	Per cent stripe
msms × Atlas	194	94	54	48	96	36
msms × Club Mariout	180	97	51	67	97	24
msms × Vaughn	184	97	12	40	98	3
msms × Hannchen	113	93	11	31	87	0
All other crosses	307	93	19	106	96	7

ment. Thus, both field and malting tests show that the stripe fungus does not reduce germination.

Effect of Hull Discoloration on Stripe Development. The hulls on seeds infected with stripe at the flowering stage commonly exhibit moderate to severe darkening at maturity. Since the technique used insures deposition of spores inside all of the hulls at flowering time, it seemed desirable to compare the gross evidence of mycelial growth within and on the hulls (discoloration) with germination and ultimate stripe expression. These data (table 2) are based on common samples, separated according to hull color. They yield further evidence that the stripe organism does not affect germination. Equally interesting is the fact that conspicuous mycelial development is about equal on all varieties, including those with genetic resistance. Ultimate incidence of the disease was in all cases significantly less in seeds with no external evidence of stripe than in those of the same stock that were darkened by mycelium. On the other hand, mycelial development coincident with kernel development did not assure ultimate high levels of stripe expression, even in susceptible hybrids.

Effect of Seeding Date on Stripe. Surveys in California in 1943 and 1944 indicated that stripe was much more prevalent and severe in fields sown early in the fall than in those sown later. Frequently, early- and late-sown fields were observed on the same farm. The same lot of seed had apparently been used in both plantings, since the same characteristic mixtures were present

in both fields. A consistent low incidence or a total absence of stripe was evident in the late-sown fields, irrespective of the stripe infection in the early-sown ones. Since stripe in other areas often occurs in spring seedlings, it seemed desirable to investigate the relation of seeding date to stripe infection.

The data (table 3) show progressive declines in stripe disease in three plantings from November to March. Elsewhere in America, where barley is sown in the spring, stripe develops despite late seeding. In the series emerging March 17, 1946 (table 3), sporulation was poor on the leaves of tillers with symptoms of stripe disease. Though a considerable number of these plants failed to head, they never exhibited either chlorotic stripes or fruiting structures. In this case, it seems probable that stripe infection retarded the development of the host plants to such an extent that the parasite was unable to complete its life cycle.

TABLE 3

EFFECT OF SEEDING DATE ON STRIPE EXPRESSION

Davis, California

Cross	Per cent stripe, 1944–45			Per cent stripe, 1945–46			Per cent of abnormal plants* 3/17/46
	Emergence date and interval			Emergence date and interval			
	12/8/44, 15 days	2/11/45, 16 days	3/15/45, 10 days	12/25/45, 20 days	1/24/46, 19 days	3/17/46, 16 days	
msms × Atlas	19	0	7	53	50	0	20
msms × Rojo	36	14	0	61	25	4	4
msms × Trebi	13	0	0	..	..	..	..
msms × Club Mariout	..	..	..	58	50	5	5
msms × Winter Tennessee	..	..	..	46	46	2	14

* Plants were dwarfed and nonheading but gave no evidence of spore development. The malformations may have resulted from stripe infection within the plant. Restricted to 3/17/46 emergence group.

Temperatures above 20° C have an inhibiting effect upon stripe development (Leukel, Dickson, and Johnson, 1933). Although soil temperature records were not available in this experiment, emergence intervals were noted carefully. The tests in 1944–45 required 10 to 16 days from seeding to emergence; those in 1945–46, from 16 to 20 days. Soil temperatures in relation to emergence interval have been reported (Leukel, Dickson, and Johnson, 1933)—a temperature of 10° C produces emergence in about 18 days, and one of 15° C in about 10 days. Thus, all seedings (table 3) appear to have germinated at temperatures well below the reported inhibiting temperature of 20° C. It seems, therefore, that factors other than soil temperature or soil moisture were operative in producing the progressive reductions in stripe. To confound the situation further, seeding date and prevailing temperature had little effect upon percentage of disease in recent laboratory inoculation experiments (Houston and Oswald, 1948).

Physiologic Specialization of the Stripe Organism. The technique for inoculation and testing of F_1 hybrids is not well suited for either testing or perpetuating cultures that may react differently on a series of host testers. Occasional natural crosses can be detected; the dispersal of spores in a contiguous area is known to be far more voluminous than that of the pollen.

Information on physiologic specialization of the stripe organism in California seemed very necessary to the breeding of resistant varieties.

A total of eight cultures of *Helminthosporium gramineum* were tested in two or more seasons, from 1944 to 1947. Originally one collection was obtained from Club Mariout (no. 1) and seven from Atlas. The latter were from widely separated fields of this variety in California.

Contamination in field cultures prevented clear-cut race identification on the host testers, which were F_1 from hybrids between *msms* and Atlas, Club Mariout, Trebi, Hannchen, or Vaughn. There was evidence, nevertheless, for two distinct races. The one, common to four of the eight cultures, was characterized by poor spore production on the hosts and relatively low levels of infection. The other involved differences in pathogenicity on the hosts. Thus, culture 6 produced a mean of only 28 per cent stripe on *msms* × Club Mariout for four seasons—an obviously low value for this cross, which regularly produces in excess of 50 per cent stripe with culture 3. With the cross *msms* × Vaughn, culture 3 had a mean of 8 per cent stripe for four seasons, but culture 8 produced 58 per cent stripe in one season. On *msms* × Trebi and *msms* × Hannchen, substantially the same resistance reactions were obtained with all cultures in all seasons.

Since the physiological specialization of the stripe organism encountered in California produced widely different infections, it seemed advisable to restrict the present genetic investigations to a single pure culture of the stripe organism.

Genetic Studies. The general nature of the resistance in the four varieties used in this experiment has already been reported (Suneson and Santoni, 1943*a*). Data presented in table 4, using culture 3, however, are conclusive in showing the nearly complete dominance of resistance in Hannchen, the partial dominance of resistance in Trebi, and the dominance of susceptibility in Club Mariout. It is further shown that when male-sterile is sibbed (*msms* × *Msms*), the progeny is not so susceptible as *msms* × Club Mariout. The male-sterile stock, therefore, possesses a weak resistance, which makes genetic evaluation of crosses with it more difficult. It should be noted also that the reaction groups—resistant, intermediate, and susceptible—in table 4 are not identical entities but are merely a device for indicating modal classes observed in each group of crosses.

The cross *msms* × Club Mariout required two backcrosses to Club Mariout to produce homozygous resistant progeny. Recombinations produced by backcrossing revealed an intermediate reaction class. This result suggests multiple gene action. In any case, resistance in Club Mariout seems to be conditioned by at least two recessive genes.

The results from the cross *msms* × Trebi point to a single gene difference, with dominance incomplete. The evidence was more positive when backcrossing the F_1 plants to susceptible male-sterile than when allowing segregation to occur in the F_2 generation and then backcrossing to Trebi. Resistance was stabilized after two backcrosses to Trebi, however.

Backcrossing male-sterile × Hannchen to the susceptible parent resulted in recovery of both intermediate and susceptible classes. Since this intermediate resistance does not show in the first generation, where dominance is complete,

it must represent a type of gene action with incomplete dominance. Preliminary test crosses with Trebi have not been conclusive, but they suggest a genetic difference. The Hannchen resistance has therefore not been fully determined, but it probably involves two or more genes.

The resistance genes herein reported have not been related to those found in Brachytic and Lion (Arny, 1945*a*).

TABLE 4

STRIPE-REACTION SEGREGATIONS IN CROSSES OF MALE-STERILE WITH THREE VARIETIES IN SEVERAL TEST GENERATIONS

Cross	Generation tested	Number of test years	Total plants grown	Type of observed reaction to stripe					
				Resistant		Intermediate		Susceptible	
				Number of lines	Stripe per cent	Number of lines	Stripe per cent	Number of lines	Stripe per cent
msms × *Msms*	F_1	4	756	0	..	0	..	24	55
msms × Club Mariout	F_1	4	296	0	..	0	..	4	69
F_2 sel. × Club Mariout	BC F_1	1	334	0	..	2	35	13	55
BC F_2 sel. × Club Mariout	BC^2F_1	1	167	1	0	4	28	0	..
BC^2F_2 sel. × F_1	$BC^2F_2 \times F_1$	2	446	5	5	10	28	0	..
msms × Trebi	F_1	3	187	0	..	3	20	0	..
msms × F_1	BC F_1	3	649	0	..	7	22	7	68
(*msms* × F_1) × F_1	BC $F_1 \times F_1$	2	818	0	..	14	33	10	55
(*msms* × F_1) × *msms*	BC^2F_1	1	639	0	..	3	32	16	60
F_2 sel. × Trebi	BC F_1	1	169	0	..	3	15	3	45
BC F_2 sel. × Trebi	BC^2F_1	1	276	8	2	0	..	0	..
msms × Hannchen	F_1	6	204	6	4	0	..	0	..
msms × F_1	BC F_1	3	1,535	12	3	24	27	6	57
(*msms* × F_1) × F_1	BC $F_1 \times F_1$	2	401	6	6	12	24	3	57

CONCLUSIONS

Complete floral inoculation of male-sterile barley with spores of the stripe-disease organism is possible. Wetting of spores and maintenance of high humidity for 6 hours after inoculation had no effect on subsequent stripe development. Under the conditions of these tests, stripe did not reduce germination.

Seeds darkened by mycelial growth coincident with development produced higher levels of stripe than did bright seeds that evidenced no spore growth prior to seed germination.

The seasonal decline from fall to spring noted in stripe expression was due to delayed seeding and was apparently independent of soil temperature or soil moisture.

At least two distinct physiologic races of the stripe organism exist in California.

Four sources of genetic resistance to stripe were recognized. These were

derived, respectively, from the varieties Hannchen, Trebi, Club Mariout, and male-sterile. Respectively, these show: dominance of resistance, partial dominance of resistance, dominance of susceptibility, and weak resistance. Collectively, at least six different genes appear to be involved.

From these and other genetic studies (Arny, 1945*a*), it is evident that resistance to the stripe disease is conditioned by a rather large number of genes. A similar broad dispersal of genes covering mildew resistance has been reported (Briggs, 1945). Further contributions on the genetics of stripe resistance, using the male-sterile technique, seem to require combining each of the several genes with the gene for male sterility. When this transfer has been accomplished, further gene identification and differentiation will be possible with relatively small populations.

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